Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Synthesis and Antimicrobial Evaluation of Bipyrimidines in Efficient Biphasic System using Zeolite as Green Catalyst

Manisha S. Aswale and Raksha P. Dhankar✉

Asian Journal of Organic & Medicinal Chemistry

Volume: 3 Year: 2018
Issue: 4 Month: October–December
pp: 143–149
DOI: https://doi.org/10.14233/ajomc.2018.AJOMC-P140

Received: 27 August 2018
Accepted: 18 November 2018
Published: 31 December 2018

ABSTRACT

A simple, green and efficient catalytic condensation process have been developed to synthesize the series of 2-amino-6-substituted-4,6-diphenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (**3a-j**) hybrids. The catalytic route was investigated efficiently in presence of NaY zeolite in organic-aqueous (dichloromethane-water) solvent system. In this method, biphasic solvent systems were explored for suitable applicability where catalyst exhibits remarkable reactivity. Synthesized compounds were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aurigenosa* and also antifungal activity against the opportunistic pathogens *Candida albicans* and *Aspergillus niger*. Among them compounds **3d**, **3e**, **3f** and **3i** exhibited very good inhibition activity for antibacterial and antifungal. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass and CHN analyses.

KEYWORDS

Bipyrimidines, Zeolite, Dichloromethane-water biphasic system, Antimicrobial activity.

INTRODUCTION

Bipyrimidines hybrids play an essential role in several biological processes and have considerable chemical and pharmacological importance. A large number of bipyrimidine derivatives are reported to antimicrobial [1], anticancer [2], antiviral [3], anti-inflammatory [4], antifungal [5], analgesic [6], anticonvulsant [7], antioxidant [8], antitubercular, antimalarial [9] and antileishmanial [10].

Zeolite X is a highly versatile molecular sieve with pore size 7.4 Å from the faujasite family of zeolites whose, three-dimensional pore structure and solid acidity make it useful as a catalyst in synthesis of analogues of several *N*-heterocycles [11,12]. Some of the special features of zeolites such as thermal stability, controlled variability, reusability and eco-friendly nature, zeolites are most required catalysts in green chemistry. Current research has depicted the efforts towards the utilization of biphasic reaction system in synthesis of variety of heterocycles. This shows remarkable importance over monophasic solvent system. The biphasic solvent system allows easy separation and reusability of the reactive aqueous phase containing spent homogeneous or heterogeneous catalysts. The recent work from various research groups indicated that biphasic

Author affiliations:

Centre for Higher Learning and Research, Department of Chemistry, Sardar Patel Mahavidyalaya, Ganjward, Chandrapur-442402, India

✉To whom correspondence to be addressed:

E-mail: rakshadhankar@rediffmail.com

Available online at: http://ajomc.asianpubs.org

reaction systems showed significant advantages in protecting the products from further degradation by extracting the products produced from the monophasic solvent, simplifying the separation steps to achieve the final products, minimizing the side reactions and increasing the overall yield [13,14].

We opted for an approach for the development of an easy, efficient, green and clean method for the synthesis of 2-amino-6-substituted-4,6-diphenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one analogues (**3a-j**). The rarity of reports in catalytic synthesis of bipyrimidines in biphasic solvent system, the work aims to study the beneficial approach in yields of bipyrimidines in DCM-water optimized phase using zeolite as a catalyst. In the present study, we have designed and synthesized a new series of bipyrimidines by simple condensation and screened for its *in vitro* antimicrobial activities where **3d**, **3e**, **3f** and **3i** were found to the exhibit the excellent potent activity as antibacterial and antifungal agents. Other compounds possessed moderate to low activity.

EXPERIMENTAL

Bipyrimidines were synthesized by using analytical grade substituted chalcone and guanidine hydrochloride (S.D. Fine Chemicals, 98 %). Zeolite, dichloromethane, ethyl acetate and *n*-hexane were obtained from Qualigen India Ltd. Mumbai.

Melting points were determined by open capillary method and are uncorrected. All solvents were distilled and dried prior to use. TLC was performed on silica gel G and the spots were exposed to iodine vapours for visualization. A mixture of *n*-hexane and ethyl acetate (7:3) was used as an eluent. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Brucker AC 400 (MHz) instrument. Chemical shifts are reported in ppm using TMS as the internal standard. IR spectra were obtained on a Perkin Elmer 1800 spectrophotometer using KBr discs and mass spectra were measured with Shimadzu gas chromatograph coupled with QP5050 Spectrometer at 1-1.5 eV. CHN elemental analysis was carried out with Perkin Elmer 300A elemental analysis.

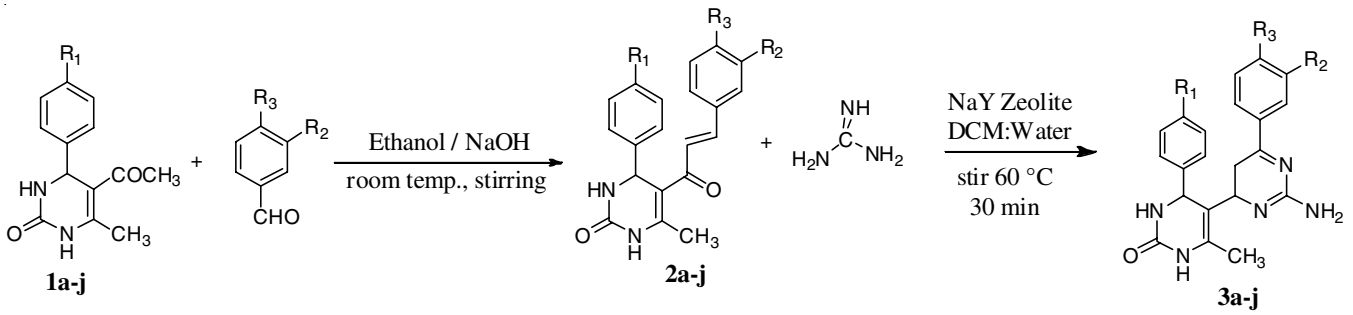
***in vitro* Antibacterial and antifungal activities:** All the series of synthesized compounds were evaluated for their efficacy against the clinically isolated microorganisms as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginonasa* (ATCC 85327) (Gram-negative bacteria) *Staphylococcus aureus* (ATCC 29213) (Gram-positive bacteria), *Candida albicans* (ATCC 102310) and *Aspergillus niger* (ATCC 439). The preliminary antimicrobial activities of the compounds **3a-j** were tested using cup-plates method [15]. The compounds to be screened were dissolved in DMSO at different concentrations *viz*. 12.5, 25, 50 and 100 µg/mL. The plates were incubated at 37 ºC for 24 h, the control was similarly maintained with 1 mL of DMSO and the zones of inhibition of bacterial and fungal growth were measured in mm. Ampicillin and ketoconazole were used as the standard drugs. The inoculated plates were incubated at 37 ºC for 24 h in the case of bacteria and 48 h in the case of fungus. The zone of inhibition was compared with the standard drugs.

The minimum inhibitory concentrations (MIC) of compounds were tested using the microdilution susceptibility method [16]. The chemical stock solutions of all the compounds and reference drugs were prepared by dissolving 1000 µg in 5 mL DMSO. A series of dilutions was prepared as 100, 50, 25 and 12.5 µg/mL. The solutions with no turbidity were considered as MIC for tested compounds.

General procedure for synthesis of 5-cinnamoyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (2a): The synthesis of chalcones was carried out *via* Claisen-Schmidt condensation. A mixture of 5-acetyl-6-methyl-4-phenyl-3,4-dihydropryimidine-2(1*H*)-one (1 mmol, 0.214 g) and benzaldehyde (1 mmol, 0.106 g) was dissolved in 10 mL of ethanol in 250 mL round bottom equipped with magnetic stirrer. Then 20 mL NaOH solution (8 g in 20 mL H_2O) was added dropwise to the reaction mixture with vigorous stirring for 0.5 h at room temperature. The reaction mixture was kept for overnight. The reaction mixture was neutralized by adding dil. HCl whereby the precipitation occurred. The product was filtered and recrystallized by ethanol. The physico-chemical analysis of the synthesized compounds are shown in Table-1.

TABLE-1
SYNTHESIZED OF SUBSTITUTED 5-CINNAMOYL-6-METHYL-4-PHENYL-3,4-DIHYDROPYRIMIDIN-2(1H)-ONE (**2a-j**)

Compd.	R_1	R_3	Yield (%)	Time (min)	m.p. (°C)
2a	-H	-H	83	90	160
2b	-H	$-NO_2$	79	120	215
2c	-H	$-OCH_3$	85	105	220
2d	$-OCH_3$	$-OCH_3$	80	110	200
2e	-Cl	-H	82	105	173
2f	-H	-Cl	82	105	168
2g	$-NO_2$	-H	85	120	210
2h	$-OCH_3$	$-NO_2$	86	115	220
2i	-Cl	$-OCH_3$	87	135	208
2j	$-NO_2$	$-OCH_3$	84	130	217



R_1 = H, NO_2, OCH_3, Cl; R_2 = H; R_3 = H, NO_2, OCH_3, ,Cl

Scheme-I

General procedure for synthesis of 2-amino-6-methyl-4,6-diphenyl-3′,4,4′,5-tetrahydro-[4,5′-bipyrimidine]-2′(1H)-one (3a): In a 50 mL of round bottom flask 5-cinnamoyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (**2a**) (5 mmol, 1.65 g) and zeolite (30 mol %, 0.573 g) were thoroughly mixed in dichloromethane and were stirred for few minutes. Guanidine hydrochloride (10 mmol, 0.95 g) dissolved in water was poured in round bottom flask of reaction mixture. The solution was now stirred in organic-water phases as reaction media at 60 ºC for 0.5 h (**Scheme-I**). The extent of the reaction was monitored by TLC. After the completion of the reaction, product was extracted from biphasic solvents as a solid material by filtration. The solvents were separated by separating funnel and water was evaporated to get the zeolite for next run. The product (**3a**) obtained was subjected to recrystallized by ethanol. The physico-chemical analysis of the synthesized compounds are shown in Table-2.

TABLE-2
SYNTHESIZED OF BIPYRIMIDINES OF 2-AMINO-6-SUBSTITUTED-4,6-DIPHENYL-3',4,4',5-TETRAHYDRO-[4,5'-BIPYRIMIDINE]-2'(1H)-ONE (**3a-j**)

Compd.	R_1	R_2	R_3	Yield (%)	Time (min)	m.p. (°C)
3a	-H	-H	-H	89	30	198
3b	-H	-H	$-NO_2$	86	34	195
3c	-H	-H	$-OCH_3$	85	36	190
3d	$-OCH_3$	-H	$-OCH_3$	84	30	222
3e	-Cl	-H	-H	87	45	202
3f	-H	-H	-Cl	83	34	188
3g	$-NO_2$	-H	-H	87	48	201
3h	$-OCH_3$	-H	$-NO_2$	86	56	265
3i	-Cl	-H	$-OCH_3$	85	47	226
3j	$-NO_2$	-H	$-OCH_3$	86	45	233

2-Amino-6-methyl-4,6-diphenyl-3′,4,4′,5-tetrahydro-[4,5′-bipyrimidine]-2′(1*H*)-one (3a): Yellow colored solid, yield: 89 % ; m.p. 198 ºC. IR (KBr, ν_{max}, cm^{-1}): 3286, 1612, 1454, 1074. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.9-7.8 (m, 10H, arom.), 8.6 (s, 1H, -NH), 8.7 (s, 1H, -NH), 3.9 (s, 2H, -NH_2), 2.4 (s, 3H, -CH_3), 2.68-2.70 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.32-3.34 (t, 1H, -CH), 5.6 (s, 2H, -CH). ^{13}C NMR ($CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 58.6, 114.4, 120.9, 127.1, 128.7, 131.0, 139.9, 141.8, 149.5, 153.6, 163.7. MS (70 eV): *m/z* = 359.17 [M^+]. Anal. calcd. (found) % for $C_{21}H_{21}N_5O$ m.w. 359.17: C, 70.17 (70.15); H, 5.89 (5.86); N, 19.48 (19.45); O, 4.45 (4.43).

2-Amino-6-methyl-6-(4-nitrophenyl-4-phenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (3b): Light yellow coloured solid, yield: 86 %, m.p. 195 ºC. IR (KBr, ν_{max}, cm^{-1}): 3285, 1611, 1450, 1072. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.8-7.2 (m, 9H, arom.), 8.1 (s, 1H, -NH), 8.3 (s, 1H, -NH), 3.9 (s, 2H, -NH_2), 2.3 (s, 3H, -CH_3), 2.58-2.60 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.32-3.34 (t, 1H, -CH), 5.6 (s, 2H, -CH). ^{13}C NMR ($CDCl_3$, δ ppm): 14.6, 35.0, 40.3, 56.4, 58.7, 115.1, 123.2, 125.2, 127.9, 128.8, 141.7, 141.8, 145.5, 149.5, 154.3, 164.7. MS (70 eV): *m/z* = 404 [M^+]. Anal. calcd. (found) % for $C_{21}H_{20}N_6O_3$, m.w. 404.14: C, 62.37 (63.65); H, 4.98 (4.97); N, 20.78 (20.75); O,11.87 (11.85).

2-Amino-6-(4-methoxyphenyl)-6-methyl-4-phenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (3c): Dark brown coloured solid, yield: 85 %. m.p. 190 ºC. IR (KBr, ν_{max}, cm^{-1}): 3282, 1613, 1452, 1072. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.9-7.4 (m, 9H, arom.), 8.5 (s, 1H, -NH), 3.9 (s, 2H, -NH_2), 2.5 (s, 3H, -CH_3), 2.83-2.85 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.22-3.24 (t, 1H, -CH), 5.4 (s, 2H, -CH), 3.2 (s, 3H, -OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 14.6, 35.1, 40.3, 56.7, 58.8, 113.1, 115.0, 124.7, 126.1, 128.5, 133.6, 141.3, 149.8, 152.3, 163.1, 165.1. MS (70 eV): *m/z* 389.19 [M^+]. Anal. calcd. (found) % for $C_{22}H_{23}N_5O_2$, m.w. 389.14: C, 67.85 (67.83); H, 5.95 (5.93); N, 17.98 (17.96); O, 8.22 (8.20).

2-Amino-4′,6-*bis*(4-methoxyphenyl)-6-methyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1H)-one (3d): Yellow coloured solid, yield: 84 %. m.p. 222 ºC. IR (KBr, ν_{max}, cm^{-1}): 3285, 1612, 1453, 1070. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.9-7.9 (m, 8H, arom.), 8.6 (s, 1H, -NH), 4.02 (s, 2H, -NH_2), 2.4 (3H, -CH_3), 2.50-2.53 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.32-3.34 (t, 1H, -CH), 5.5 (s, 2H, -CH), 3.6 (s, 3H, -OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm): 14.1, 35.1, 39.8, 55.9, 58.8, 114.2, 115.0, 124.6, 126.7, 128.9, 133.2, 139.9, 141.8, 149.5, 152.8, 159.9, 162.4, 164.1. MS (70 eV): *m/z* 419.19 [M^+]. Anal. calcd. (found) % for $C_{23}H_{25}N_5O_3$, m.w. 419.18: C, 65.85 (65.82); H, 6.01 (6.01); N, 16.70 (16.68); O, 11.44 (11.42).

2-Amino-4-(4-chlorophenyl)-6-methyl-6-phenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (3e): Pale yellow coloured solid, yield: 87 %, m.p. 202 ºC. IR (KBr, ν_{max}, cm^{-1}): 3281, 1610, 1452, 1073. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.3-7.9 (m, 9H, arom.), 8.6 (s, 1H, -NH), 3.9 (s, 2H, -NH_2), 2.1 (s, 3H, -CH_3), 2.60-2.63 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.42-3.44 (t, 1H, -CH), 5.6 (s, 2H, -CH). ^{13}C NMR ($CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 58.6, 114.4, 123.5, 127.1, 128.3, 129.8, 131.3, 139.9, 141.8, 149.5, 153.6, 163.7. MS (70 eV): *m/z* = 393.14 [M^+]. Anal. calcd. (found) % for $C_{21}H_{20}N_5OCl$, m.w. 393.12: C, 64.04 (64.02); H, 5.12 (5.10); N, 17.78 (17.75); O, 4.06 (4.03); Cl, 9.00 (9.90).

2-Amino-6-(4-chlorophenyl)-6-methyl-4-phenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (3f): Light yellow coloured solid, yield: 83 %; m.p. 188 ºC. IR (KBr, ν_{max}, cm^{-1}): 3283, 1612, 1454, 1073. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.4-7.8 (m, 9H, arom.), 8.7 (s, 1H, -NH), 3.7(s, 2H, -NH_2), 2.1 (s, 3H, -CH_3), 2.40-2.43 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.32-3.34 (t, 1H, -CH), 5.7 (s, 2H, -CH). ^{13}C NMR ($CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 58.6, 114.4, 123.6, 127.1, 128.7, 131.0, 135.9, 137.6, 141.8, 149.5, 153.6, 163.7. MS (70 eV): *m/z* = 393.14 [M+]; Anal. calcd. (found) % for $C_{21}H_{20}N_5OCl$, m.w. 393.14: C, 64.04 (64.02); H, 5.12 (5.10); N, 17.78 (17.75); O, 4.06 (4.03); Cl, 5.12 (5.10).

2-Amino-6-methyl-4-(4-nitrophenyl)-6-phenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (3g): Yellow coloured solid, yield: 87 %, m.p. 201 ºC. IR (KBr, ν_{max}, cm^{-1}): 3284,1610,1453,1072. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.5-7.9 (m, 9H, arom.), 8.6 (s, 1H, -NH), 8.1 (s, 1H, -NH), 3.9 (s, 2H, -NH_2), 2.2 (3H, -CH_3), 2.47-2.50 (d, *J* = 10.68 Hz, 2H, -CH_2), 3.34-3.32 (t, 1H, -CH), 5.6 (s, 2H, -CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 58.6, 114.4, 123.6, 123.9, 127.1, 128.7, 131.0, 139.9, 146.2, 147.9, 149.5, 153.6, 163.7. MS (70 eV): *m/z* 404.16 [M^+]. Anal. calcd. (found) %

for $C_{21}H_{20}N_6O_3$, m.w. 404.16: C, 62.37 (62.35); H, 4.98 (4.97); N, 20.78 (20.75); O, 11.87 (11.85).

2-Amino-4-(4-methoxyphenyl)-6-methyl-6-(4-nitrophenyl)-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1H)-one (3h): Dark yellow solid, yield: 86 %, m.p. 265 ºC. IR (KBr, ν_{max}, cm^{-1}): 3286, 1613, 1453, 1073. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.7-7.8 (m, 8H, arom.), 8.6 (s, 1H, -NH), 8.2 (s, 1H, -NH), 4.0 (s, 2H, $-NH_2$), 2.2 (s, 3H, $-CH_3$), 2.50-2.53 (d, *J* = 10.68 Hz, 2H, $-CH_2$), 3.42-3.44 (t, 1H, -CH), 5.6 (s, 2H, -CH), 3.7 (s, 3H, $-OCH_3$). ^{13}C NMR ($CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 58.6, 113.1, 114.4, 123.4, 125.5, 127.1, 128.7, 135.7, 146.9, 149.5, 153.6, 158.5, 163.7. MS (70 eV): *m/z* 434.45 [M^+]. Anal. calcd. (found) % for $C_{22}H_{22}N_6O_4$, 434.42: C, 60.87 (60.85); H, 5.10 (4.98); N, 19.34 (19.32); O, 14.73 (14.70).

2-Amino-6-(4-chlorophenyl)-6-methyl-4′-(4-methoxyphenyl)-6′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1H)-one (3i): Yellow coloured solid, yield: 85 %, m.p. 226 ºC. IR (KBr, ν_{max}, cm^{-1}): 3286, 1614, 1453, 1074. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.0-7.9 (m, 8H, arom.), 8.6 (s, 1H, -NH), 8.0 (s, 1H, -NH), 3.9 (s, 2H, $-NH_2$), 2.3 (s, 3H, $-CH_3$), 2.50-2.53 (d, *J* = 10.68 Hz, 2H, $-CH_2$), 3.32-3.34 (t, 1H, -CH), 5.4 (s, 2H, -CH), 3.7 (s, 3H, $-OCH_3$). ^{13}C NMR ($CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 56.2, 58.6, 113.9, 114.4, 115.8, 123.7, 124.9, 127.1, 128.7, 133.1, 136.5, 138.9, 149.5, 153.6, 158.4, 163.7. MS (70 eV): *m/z* 423.18 [M^+]. Anal. calcd. (found) % for $C_{22}H_{22}N_5O_2Cl$, m.w. 423.18: C, 65.17 (65.15); H, 5.72 (5.73); Cl, 8.36 (8.00); N, 17.27 (17.29); O, 8.84 (7.85).

2-Amino-4′-(4-methoxyphenyl)-6′-methyl-6′-(4-nitrophenyl)-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1H)-one (3j): Light yellow solid, yield: 86 %, m.p. 233 ºC. IR (KBr, ν_{max}, cm^{-1}): 3282, 1610, 1452, 1074. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.7-8.2 (m, 8H, arom.), 8.5 (s, 1H, -NH), 8.5 (s, 1H, -NH), 4.0 (s, 2H, $-NH_2$), 2.2 (s, 3H, $-CH_3$), 2.50-2.53 (d, *J* = 10.68 Hz, 2H, $-CH_2$), 3.42-3.44 (t, 1H, -CH), 5.6 (s, 2H, -CH), 3.7 (s, 3H, $-OCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 15.2, 35.8, 40.9, 56.2, 58.6, 113.9, 114.4, 115.8, 124.9, 127.1, 128.7, 133.9, 135.7, 149.5, 153.6, 163.7, 165.2. MS (70 eV): *m/z* 423.16 [M^+]. Anal. calcd. (found) % for $C_{22}H_{22}N_5O_2Cl$, m.w. 423.16: C, 62.34 (62.36); H, 5.23 (5.25); N, 16.52 (16.53); Cl, 8.36 (8.35); O, 7.55 (7.55).

RESULTS AND DISCUSSION

In a model reaction of 5-cinnamoyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (**2a**) and guanidine hydrochloride in biphasic system of solvents of DCM and water were subjected to catalytic reaction in presence of NaY zeolite for the synthesis of 2-amino-6-methyl-4,6-diphenyl-3′,4,4′,5-tetrahydro[4,5′-bipyrimidine]-2′(1*H*)-one (**3a**) at 60 ºC for 30 min stirring.

Initially, our efforts focused on delineating a simple, green condensation reaction of 5-cinnamoyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (**2a**) and guanidine hydrochloride in presence of NaY zeolite to get 2-amino-6-methyl-4,6diphenyl-3′,4,4′,5-tetrahydro-[4,5′-bipyrimidine]-2′(1H)-one (**3a**). We proceeded with an organic solvent of dichloromethane which out came with only 12 % yield. Further we worked on the reaction with other aprotic solvents as *n*-hexane, toluene, cyclohexane, ethyl acetate and THF, results were unsuccessful. Looking into the disadvantages of monophasic solvents system, we focused our efforts on the organic-aqueous biphasic system to get better yields of product [17-19]. The biphasic solvents play a crucial role in determining the amount of product in catalysis. The first step towards determining the biphasic organic solvents that are immiscible with water, results in minimum number of toxic side products and intermediates with clean environmental approach [20] (Table-3). All other permutations generated by varying parameters, such as concentration of zeolite, reaction time for condensation, reaction temperature; ratio of organic-aqueous system led to lower yields. The extensive optimization for the various biphasic system (Table-3), DCM-water 1:1 ratio (Table-4) in 30 mol % of catalyst (Table-5) furnished astonishing results with 89 % in yield. The possible explanation of such an amazing behaviour of organic-aqueous reaction media of DCM-water system is due to characteristic properties of DCM as compared to other solvents *i.e.* high polarity, high dielectric constant, high dipole moment and low boiling point of dichloromethane.

TABLE-3
OPTIMIZATION OF VARIOUS ORGANIC-AQUEOUS SOLVENT SYSTEMS

Entry No.	Solvent system	Time (min)	Yield (%)	Ratio Organic:Aqueous
1	Toluene-water	60	30	7:3
2	DCM-water	30	45	7:3
3	Cyclohexane-water	70	25	7:3
4	*n*-Hexane-water	66	23	7:3
5	Ethylacetate-water	80	22	7:3
6	DMA-water	75	30	7:3

TABLE-4
OPTIMIZATION OF RATIO OF DCM-WATER SYSTEM

S. No.	Organic solvent (DCM) (mL)	Water (mL)	Temp. (°C)	Yield (%)	Time (min)
1	7	4	60	50	45
2	7	5	60	56	42
3	7	6	60	75	40
4	7	7	60	89	30

TABLE-5
OPTIMIZATION OF CATALYST CONCENTRATIONS FOR SYNTHESIS OF BIPYRIMIDINES (**3a-j**)

S. No.	Catalyst conc. (mmol %)	Reaction time (min)	Yield (%)
1	10	120	32
2	12.5	115	35
3	16	100	45
4	20	90	47
5	22.5	75	56
6	25	50	87
7	30	30	89

Table-4 showed that the organic solvent and water ratio had a significant impact on the product yield. To understand the effect of organic phase on the yield of product, distribution of different ratio of biphasic system was carried out during the reaction. Initially, only in monophasic system gave we could obtain 12 % yield which was very low. However, using the different ratio of DCM-water system with zeolite, the yield

obtained was dramatically enhanced. When the proportion ratio reached at 1:1, it was observed that the reaction yield was greatest. Further increasing the organic solvent ratio resulted in the decrease of yield. It is interesting to note that the amount of product was reduced when the reaction was carried in pure organic phase and when the reaction mixture was diluted with aqueous phase the yield was augmented which indicated that water is essential for this reaction.

The purity of compound was confirmed by a single spot in TLC. In IR spectra of compound **2a** carbonyl absorption at 1722-1690 cm^{-1} and -NH absorption at 3225 cm^{-1} have been observed. The compound showed mass ion peak of 100 % intensity corresponding to its molecular weight in mass spectra further confirmed the structure. Furthermore, ^1H NMR spectra of compound **2a**, a singlet appeared at δ 2.12 ppm owing to three proton of -CH_3. A doublet at δ 6.93 to δ 6.88 ppm is assigned for one proton of -CO-CH= group of chalcone with (J = 17.8 Hz). The peak of doublet at δ 7.46 to δ 7.40 ppm represents for one proton of =CH-Ar with (J = 17.12 Hz). A singlet for one proton of -NH appears at δ 8.32 ppm and singlet of one proton of -CH of Biginelli ring corresponds at δ 5.50 ppm. A singlet for one protons of -NH appears at δ 8.23 ppm. The multiplet peaks at δ 7.6 to δ 6.9 corresponds to 10 aromatic protons indicating the presence of two phenyl rings. The product **2a** was analyzed for $C_{20}H_{18}N_2O_2$ which exhibited molecular ion at m/z = 318.14 [M+1].

The spectra of hybrid of synthesized compounds (**3a-j**) were analyzed by ^1H NMR, IR and mass spectra. In IR spectra of compound **3a** exhibited carbonyl absorption band at 1722-1612 cm^{-1} and -NH stretching band at 3286 cm^{-1}. The ^1H NMR spectra of compound **3a**, a singlet appeared at δ 2.4 ppm owing to three proton of -CH_3.The multiplet resonated at δ 7.8 to δ 6.9 ppm corresponds to 10 aromatic protons indicating the presence of two phenyl rings. The triplet peak at δ 3.34-3.32 indicated presence of -CH group and doublet appeared at δ 2.70-2.68 ppm represented presence of -CH_2 group of primidine ring of compound **3a**. Similarly, the peak of -NH observed at δ 8.6 ppm and the peak of -NH appeared at δ 8.7 ppm respectively. The singlet peak of -NH_2 showed at δ 3.9 ppm. A singlet at δ 5.6 ppm indicated presence of -CH group of Biginelli compound. The product **3a** was analyzed for $C_{21}H_{21}N_5O$ which exhibited molecular ion at m/z = 359.17 [M+1].

Antimicrobial evaluation: All the synthesized compounds were screened as potent antibacterial and antifungal scaffolds **3a-j**. The microbial study was carried out against two Gram negative bacterial strains namely *E. coli* (ATCC 25922) and *P. aeruginonasa* (ATCC 8532) and one Gram positive bacterial strain named *S. aureus* (ATCC 29213) and against two fungal strains namely as *C. albicans* (ATCC 10231) and *A. niger* (ATCC 439). The screening was performed with the standard drugs as ampicillin for antibacterial activity and ketoconazole for antifungal activity. The microbial activity of bipyrimidine derivatives were further evaluated for the minimum inhibition concentration (MIC). The inhibitions of microbial growth were used to demonstrate the therapeutic efficacy of hybrid scaffolds. The activity data is illustrated in Tables 6-8. The results of microbial analysis of the tested compounds revealed that these hybrids have shown moderate to good antibacterial efficacy against selected bacteria strains (*E. coli*, *P. aeruginosa* and *S. aureus*). On the basis of zone of inhibition test against test bacterium, *E. coli*, compounds **3i** (R_1 = Cl, R_3 = OCH_3), **3e** (R_1 = Cl, R_3 = H) and **3f** (R_1 = H, R_3 = Cl) were found to have very good activity; and compounds **3d** (R_1 = OCH_3, R_3 = OCH_3), **3c** (R_1 = H, R_3 = OCH_3 and **3a** (R_1 = H, R_3 = H) possessed good activity, while compound **3j** (R_1 = Cl, R_3 = OCH_3), **3h** (R_1 = OCH_3, R_3 = NO_2), **3g** (R_1 = NO_2, R_3 = H), **3b** (R_1 = H, R_3 = NO_2) showed moderate activity when compared with the standard drug ampicillin. In case of *P. aeruginosa*, compounds **3i** and **3f** very good activity and compounds **3a**, **3c**, **3d**, **3e** and **3j** exhibited the good activity while compounds **3b**, **3g** and **3h** resulted into moderate activity as compared to the standard drug ampicillin. For *S. aureus*, compounds **3e**, **3f** and **3i** showed very good activity, compounds **3a**, **3c**, **3d** and **3j** possessed good activity while compounds **3b**, **3g** and **3h** revealed moderate activity in comparison to the standard drug ampicillin.

For fungal strains, the screening of synthesized compounds have revealed from very good activity to moderate activity. For *Candida albicans*, compounds **3f** and **3i** showed very good activity and compounds **3c** and **3e** possessed good activity while compounds **3a**, **3b**, **3d**, **3g**, **3h** and **3i** exhibited moderate activity

TABLE-6
INHIBITION ZONES (mm) OF SYNTHESIZED BIPYRIMIDINES (**3a-j**) AGAINST BACTERIA AND FUNGI BY THE DISK DIFFUSION METHOD

Compound	Microbial							Fungal			
	Escherichia coli (ATCC 25922)			*P. aeruginonas* (ATCC 8532)	*Staphylococcus aureus* (ATCC 29213)			*Candida albicans* (ATCC 10231)		*Aspergillus niger* (ATCC 439)	
	50 µg	25 µg	12.5 µg	100 µg	100 µg	50 µg	25 µg	100 µg	50 µg	100 µg	50 µg
3a	11	12	10	11	12	12	10	10	07	10	07
3b	10	11	09	–	11	09	–	10	08	09	06
3c	12	12	10	12	12	12	10	12	09	11	09
3d	12	11	10	11	13	12	10	11	07	12	09
3e	14	12	10	12	14	14	11	13	09	14	09
3f	14	12	11	13	14	13	11	14	10	15	09
3g	10	11	08	06	10	08	–	11	08	10	05
3h	10	11	09	07	11	10	–	11	07	09	06
3i	15	12	11	13	15	14	12	15	11	15	10
3j	10	10	09	11	12	12	–	11		08	06
Ampicillin	18	15	12	16	32	29	24	–	–	–	–
Ketoconazole	–	–	–	–	–	–	–	20	12	20	12

TABLE-7
MINIMUM INHIBITORY CONCENTRATIONS (μg/mL) OF SYNTHESIZED **3a-j** AGAINST AND GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Entry	*Escherichia coli* (ATCC 25922)					*Pseudomonas aeruginonasa* (ATCC 8532)					*Staphylococcus aureus* ATCC 29213)				
	12.5	25	50	100	App MIC	12.5	25	50	100	App MIC	12.5	25	50	100	App MIC
3a	+	+	++	++	50	–	++	++	++	25	–	+	++	++	50
3b	+	+	+	++	100	–	–	++	++	50	–	+	++	++	50
3c	+	++	++	+++	25	–	++	++	++	25	–	+	++	++	50
3d	+	++	++	++	50	–	++	++	+++	25	–	+	++	++	100
3e	+	++	++	++	25	–	++	++	++	50	–	+	++	++	50
3f	+	++	++	+++	25	–	++	++	+++	25	–	++	++	+++	25
3g	+	+	+	++	100	–	–	+	++	100	–	+	+	++	100
3h	+	+	+	++	100	–	–	+	++	100	–	+	+	++	100
3i	+	++	++	+++	25	–	++	++	+++	25	–	++	++	+++	25
3j	+	+	+	++	100	–	–	+	++	100	–	–	++	++	50
Ampicillin	+++	+++	+++	+++		+++	+++	+++	+++		+++	+++	+++	+++	

Symbols: (–) Confluent growth (no inhibition), Inactive (< 10 mm); (+) weakly active (07–10 mm); (++) moderately active (11–15 mm); (+++) highly active (18–23 mm).

TABLE-8
MINIMUM INHIBITORY CONCENTRATIONS (μg/mL) OF SYNTHESIZED **3a-j** AGAINST FUNGI

Entry	*Candida albicans* ATCC (10231)					*Aspergillus niger* ATCC (439)				
	12.5	25	50	100	App MIC	12.5	25	50	100	App MIC
3a	–	+	++	++	50	–	+	+	++	100
3b	–	–	++	++	50	–	–	++	++	50
3c	–	++	++	++	25	–	++	++	++	25
3d	–	+	++	++	50	–	–	+	++	100
3e	–	++	++	++	25	–	++	++	++	25
3f	–	++	++	++	25	–	++	++	++	25
3g	–	–	–	++	100	–	–	+	++	100
3h	–	–	+	++	100	–	–	+	++	100
3i	–	++	++	++	25	–	++	++	++	25
3j	–	–	+	++	100	–	–	–	++	100
Ketoconazole	+++	+++	+++	+++		+++	+++	+++	+++	+++

Symbols: (–) Confluent growth (no inhibition), Inactive (< 10 mm); (+) weakly active (07–10 mm); (++) moderately active (11–15 mm); (+++) highly active (18–23 mm)

as compared to the standard drug ketoconazole. In case of *A. niger*, compounds **3e** and **3i** revealed very good activity and compounds **3a**, **3c**, **3d**, **3f** and **3g** possessed the good activity while compounds **3b**, **3h** and **3j** displayed the moderate zone of inhibition.

Antibacterial activity: For evaluation of microbial activity, as minimum inhibition concentration of the synthesized compounds were studied at different concentrations as 12.5, 25, 50, 100 μg/mL. Among these compounds **3c**, **3e**, **3f** and **3i** exhibited very good inhibition at MIC = 25 μg/mL. Compounds **3a** and **3d** showed good activity with MIC = 50 μg/mL and the compounds **3b**, **3g**, **3h** and **3j** revealed moderate activity with MIC = 100 μg/mL against *E. coli*.

Against *P. aeruginonasa*, compounds **3d**, **3f** and **3i** exhibited very good inhibition as MIC= 25 μg/mL. Compounds **3b**, **3c**, and **3e** compounds revealed good inhibition with MIC = 50 μg/mL and compounds **3a**, **3g**, **3h** and **3j** resulted MIC = 100 μg/mL. The compounds **3f** and **3i** exhibited very good inhibition at MIC = 25 μg/mL, compounds **3a**, **3c** and **3e** compounds showed good activity with MIC = 50 μg/mL and compounds **3b**, **3d**, **3g**, **3h** and **3j** revealed moderate activity with MIC = 100 μg/mL against *S. aureus*.

Antifungal activity: Minimum inhibition concentration of all the derivatives of bipyrimidines for antifungal activity were studied and the results of compounds **3c**, **3e**, **3f** and **3i** exhibit very good inhibition by way of MIC = 25 μg/mL. Compounds **3a**, **3b** and **3d** compounds revealed good inhibition with MIC = 50 μg/mL and compounds **3g**, **3h** and **3j** moderate inhibition MIC = 100 μg/mL against *C. albicans*. In the case of *A. niger* MIC = 25 μg/mL exhibit very good inhibition of compounds **3c**, **3e**, **3f** and **3i**. The compounds **3b** showed good inhibition with MIC = 50 μg/mL and compounds **3a**, **3d**, **3g**, **3h** and **3j** revealed moderate inhibition with MIC = 100 μg/mL.

The results depicted in Tables 7 and 8 suggested that the electron withdrawing substituents revealed very significant minimum inhibition concentration whereas electron donating substitutents exhibited insignificant minimum inhibition concentration activity against microbial strains.

Conclusion

An expedite process catalyzed by zeolite has the merit of being an environmentally friendly simple operation, involving convenient workup, a short reaction time and resulting in good to excellent yields. Substituted 2-amino-6-methyl-4,6-diphenyl-3′,4,4′,5-tetrahydro-[4,5′-bipyrimidine]-2′(1*H*)-one synthesized by substituted chalcone and guanidine hydrochloride in biphasic system were confirmed by spectral characterization. The synthesized scaffolds of bipyrimidines were studied as anti-

microbial agents. The investigation of antimicrobial screening data revealed that among 10 compounds screened, compounds **3d**, **3e**, **3f** and **3i** demonstrated very good activity as compared to standard drugs and remaining compounds showed good to moderate inhibition activities.

ACKNOWLEDGEMENTS

The authors are thankful to Sophisticated Analytical Instrumentation Facility (SAIF), Chandigarh, India for ^{1}H NMR, ^{13}C NMR, IR, mass and CHN spectroscopic analysis and The Microcare Laboratory and Tuberculosis Research Centre, Surat, India for antimicrobial activities.

REFERENCES

1. S. Jain, P.K. Paliwal, G. Neelaiah Babu and A. Bhatewara, DABCO Promoted One-Pot Synthesis of Dihydropyrano(*c*)chromene and Pyrano-[2,3-*d*]pyrimidine Derivatives and Their Biological Activities, *J. Saudi Chem. Soc.*, **18**, 535 (2014); https://doi.org/10.1016/j.jscs.2011.10.023.
2. W. Zhu, C. Sun, S. Xu, C. Wu, J. Wu, M. Xu, H. Zhao, L. Chen, W. Zeng and P. Zheng, Design, Synthesis, Anticancer Activity and Docking Studies of Novel 4-morpholino-7,8-dihydro-5*H*-thiopyrano[4,3-*d*]-pyrimidine Derivatives as mTOR Inhibitors, *Bioorg. Med. Chem.*, **22**, 6746 (2014); https://doi.org/10.1016/j.bmc.2014.11.003.
3. M. Ivanov and L. Aleksandrova, Bicyclic Furano-, Pyrrolo-, and Thiopheno[2,3-d] Derivatives of Pyrimidine Nucleosides: Synthesis and Antiviral Properties, *Russ. J. Bioorg. Chem.*, **39**, 22 (2013); https://doi.org/10.1134/S1068162013010044.
4. M. Alam, M. Akhter Husain, A. Marella, O. Tanwar, R. Ali, S. Hasan, H. Kumar, R. Haider and M. Shaquiquzzaman, Anti-Inflammatory and Antimicrobial Activity of 4,5-Dihydropyrimidine-5-carbonitrile Derivatives and their Synthesis and Spectral Elucidation, *Acta Pol. Pharm.*, **69**, 1077 (2012).
5. S. Bari and N. Haswani, Design, Synthesis and Molecular Docking Study of Thienopyrimidin-4(3H)thiones as Antifungal Agents, *J. Saudi Chem. Soc.*, **21**, S264 (2017); https://doi.org/10.1016/j.jscs.2014.02.011.
6. M. Ashour, O. Shaaban, O. Rizk and I. El-Ashmawy, Synthesis and Biological Evaluation of Thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4]-triazines and Thieno[2,3-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines as Anti-inflammatory and Analgesic Agents, *Eur. J. Med. Chem.*, **62**, 341 (2013); https://doi.org/10.1016/j.ejmech.2012.12.003.
7. M. Shaquiquzzaman, S. Khan, M. Amir and M. Alam, Synthesis, Anti-convulsant and Neurotoxicity Evaluation of Some New Pyrimidine-5-carbonitrile Derivatives, *J. Saudi Pharm.*, **20**, 149 (2012); https://doi.org/10.1016/j.jsps.2011.09.007.
8. P. Attri, R. Bhatia, J. Gaur, B. Arora, A. Gupta, N. Kumar and E. Choi, Triethylammonium Acetate Ionic Liquid Assisted One-Pot Synthesis of Dihydropyrimidinones and Evaluation of their Antioxidant and Antibacterial Activities, *Arab. J. Chem.*, **10**, 206 (2017); https://doi.org/10.1016/j.arabjc.2014.05.007.
9. A. Chaudhary, P. Sharma, P. Verma and R. Dudhe, Synthesis of Novel Pyrimidine Derivative and its Biological Evaluation, *Anal. Univ. din Bucuresti-Chim.*, **20**, 123 (2011).
10. S.N. Suryawanshi, S. Kumar, R. Shivahare, S. Pandey, A. Tiwari and S. Gupta, Design, Synthesis and Biological Evaluation of Aryl Pyrimidine Derivatives as Potential Leishmanicidal Agents, *Bioorg. Med. Chem. Lett.*, **23**, 5235 (2013); https://doi.org/10.1016/j.bmcl.2013.06.060.
11. V.V.K. Mohan Kandepi and N. Narender, Synthesis of N-Heterocyclic Compounds Over Zeolite Molecular Sieve Catalysts: An Approach towards Green Chemistry, *Catal. Sci. Technol.*, **2**, 471 (2012); https://doi.org/10.1039/C2CY00162D.
12. Y. Ni, A. Sun, X. Wu, G. Hai, J. Hu, T. Li and G. Li, The Preparation of Nano-Sized H[Zn, Al]ZSM-5 Zeolite and Its Application in the Aromatization of Methanol, *Micropor. Mesopor. Mater.*, **143**, 435 (2011); https://doi.org/10.1016/j.micromeso.2011.03.029.
13. M.B. Bushuev, D.P. Pishchur, V.A. Logvinenko, Y.V. Gatilov, I.V. Korolkov, I.K. Shundrina, E.B. Nikolaenkovac and V.P. Krivopalov, A Mononuclear Iron(II) Complex: Cooperativity, Kinetics and Activation Energy of the Solvent-Dependent Spin Transition, *Dalton Trans.*, **45**, 107 (2016); https://doi.org/10.1039/C5DT03750F.
14. M. Palucki, Pyrimidines, Tetrahedron Organic Chemistry Series, vol. 26, Chap. 11, pp. 475-509 (2007); https://doi.org/10.1016/S1460-1567(07)80060-2.
15. C.H. Collins, P.M. Lyne, J.M. Grange and J.O. Falkinham III, Collins and Lyne's Microbiological Methods, Arnold, a Member of the Hodder Headline Group: London, edn 8 (2004).
16. J. Yao and R. Moellering, eds. P. Murray, E. Baron, M. Pfaller, F. Tenover and R. Yolken: Antibacterial Agents, In: Manual of Clinical Microbiology, ASM, Washington DC, pp. 1281-1290 (1995).
17. T.F. Wang, M.W. Nolte and B.H. Shanks, Catalytic Dehydration of C6 Carbohydrates for the Production of Hydroxymethylfurfural (HMF) as a Versatile Platform Chemical, *Green Chem.*, **16**, 548 (2014); https://doi.org/10.1039/C3GC41365A.
18. G. Tian, X. Tong, Y. Cheng and S. Xue, Tin-Catalyzed Efficient Conversion of Carbohydrates for the Production of 5-Hydroxymethyl-furfural in the Presence of Quaternary Ammonium Salts, *Carbohydr. Res.*, **370**, 33 (2013); https://doi.org/10.1016/j.carres.2013.01.012.
19. S. Crossley, J. Faria, M. Shen and D.E. Resasco, Solid Nanoparticles that Catalyze Biofuel Upgrade Reactions at the Water/Oil Interface, *Science*, **327**, 68 (2010); https://doi.org/10.1126/science.1180769.
20. S. Csihony, L.T. Mika, G. Vlad, K. Barta, C.P. Mehnert and I.T. Horvath, Oxidative Carbonylation of Methanol to Dimethyl Carbonate by Chlorine-Free Homogeneous and Immobilized 2,2′-Bipyrimidine Modified Copper Catalyst, *Coll. Czech. Chem. Commun.*, **72**, 1094 (2007); https://doi.org/10.1135/cccc20071094.